

Definition

Exfoliative cytopathology—the Papanicolaou method, or Pap test—is the study of normal and disease-altered, spontaneously exfoliated, or mechanically dislodged cells (surface microbiopsy) for the detection and diagnosis of various infections, abnormal hormonal activities, and precancerous or cancerous lesions.

Technique

The cytopathologist can give an accurate interpretation only if the cellular sample is adequate and well preserved. He renders a disservice to the patient if he accepts and interprets a poorly obtained specimen.

The following material is needed:

- A glass pipette with a rubber bulb
- Spatula or diagonally cut tongue depressor, preferably made of wood (the cells have a tendency to slide off the surface of a smooth plastic or metal instrument)
- Glass slides with one frosted end
- A container with saline
- A container with 95% ethanol or a spray fixative

A number of precautions can be taken to ensure that specimens are correctly obtained and processed. First, before taking the specimen, the name and identification number of the patient should be clearly written on the frosted end of the slides with a lead pencil. Each smear taken from each patient should be placed in an individual bottle of fixative to prevent possible cellular cross-contamination or mixup of specimens. The properly identified requisition should be completed to include a brief history of the patient with, at least, her age and the date of her last menstrual period recorded.

All talcum powder should be washed away from the gloves before touching the patient, instruments, or slides. A contaminant talcum crystal on a smear may hide the only diagnostic cell of the specimen! The speculum should be introduced without lubricant. Saline solution may be used for moistening. The lubricant does not dissolve during processing of the slide. It often stains dark purple and may hide numerous cells. Before scraping, the wooden spatula should be dipped in saline to moisten it and prevent too rapid dehydration of the scraped cells. When smeared, the slide should be dropped *immediately* into 95% ethyl alcohol or sprayed before it has a chance to air dry. An adequate amount of spray should be used to cover the whole surface with a thick coating.

If the slide is to be mailed, it should remain in ethanol for at least 30 minutes, but no longer than 48 hours. It is

then removed, air dried, and placed in a cardboard mailer. If sprayed, the coat of fixative should be allowed to dry completely before closing the cover of the cardboard mailer; otherwise the slide may stick to the surface of the dry cardboard, which will absorb part of the cellular moisture. This may result in unsatisfactory, air-dried, and distorted cells. Furthermore, the chance of breakage of the slide during transfer is increased. If the smear is accidentally allowed to air dry, do not place it in a fixative. Mail it as is. Most of the cells can be rehydrated in the lab if the air-drying cellular distortions have not been fixed forever!

Besides the preceding general approach, some additions or variations may be required depending on the purpose of the test. For a routine Pap smear to detect occult cervical lesions (Figure 178.1), several drops of endocervical secretions are obtained either by aspiration with the glass pipette fitted with a rubber bulb or by using the wooden spatula with a special elongated tip. These drops are placed in the middle of the prepared slide and *not* smeared. The small end of the spatula is introduced into the endocervical os, as deeply as possible, and rotated 360 degrees while energetically scraping the entire surface of the external os and part of the endocervical canal mucosa. In all postmenopausal women or in cases when an endometrial lesion is suspected, a few droplets of vaginal pool secretion should be added to this mixture before smearing. Immediately drop the smeared slide gently into the bottle of 95% ethanol fixative or spray it before it has a chance to air dry.

For diagnosis of visible, ulcerated, or fungating cervical lesions, the margins, not the top, of a fungating tumor or the bottom of an ulcerated lesion should be energetically scraped. Otherwise, most often the specimen will contain only nondiagnostic serum precipitate, cellular debris, and inflammatory cells. The occasional malignant cells are usually too degenerated for proper interpretation. A vaginal pool secretion smear is often more rewarding than an endocervical aspiration smear in such cases.

When a Pap test is done for hormonal evaluation, the healthy and intact surface of the lateral wall of the upper third of the vagina should be *gently* scraped, smeared, and fixed immediately. The smear should not be taken from an ulcerated or grossly inflamed area or from the cervix.

The Pap test is also used for detection of endometrial lesions. The pickup rate of endometrial adenocarcinoma by the use of the routine, cervical scraping technique is only about 50%. This degree of detection reaches 90% if the endometrial cavity is sampled by any instrument that crosses the endocervical mucous plug and dislodges endometrial cells. The nondisposable Killian antrum cannula and Isaac's Endometrial Cell Sampler and the disposable Mi-Mark Helix or Muenzer Endometrial Device or washing with saline of the endometrial cavity (Gravlee Jet Washing) can all produce excellent endometrial cellular samples in experienced and careful hands.

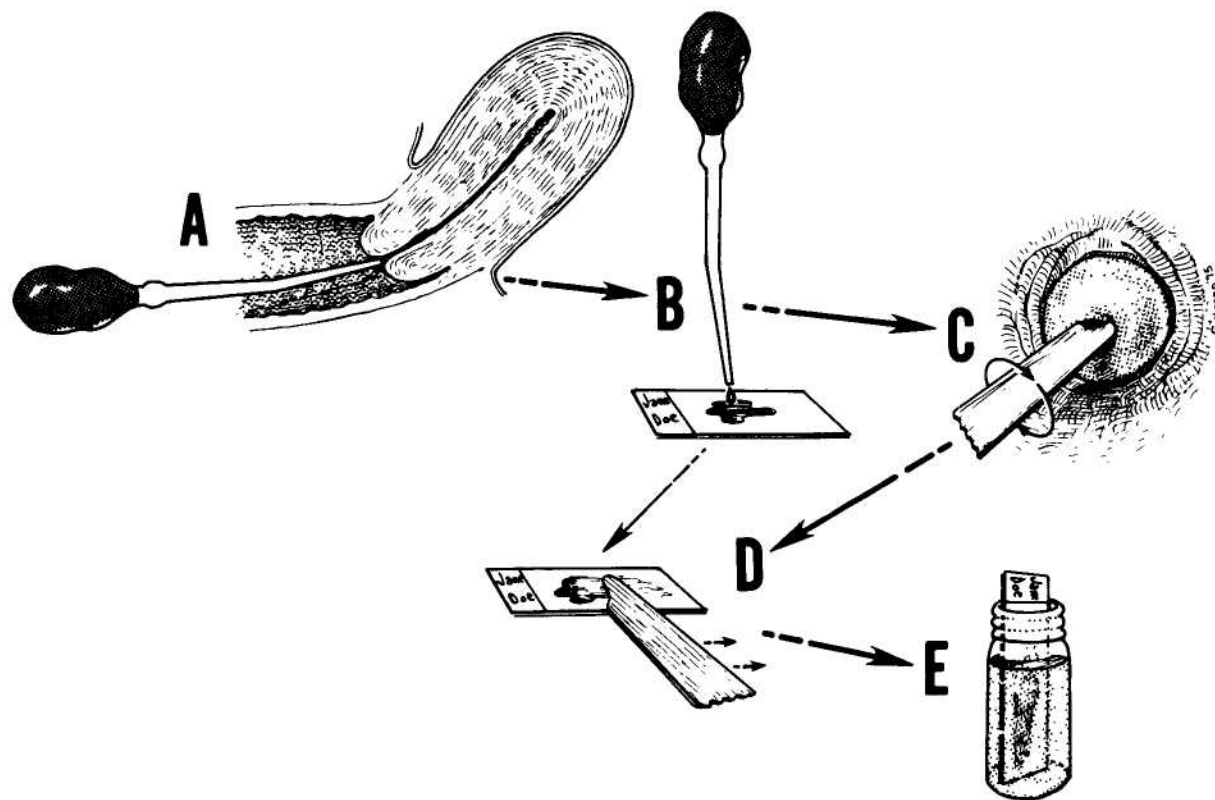


Figure 178.1

Pap test technique (A) Several drops of endocervical secretion are aspirated. (B) The drops are placed on a labeled slide and not smeared. (C) The cervix and endocervical os are scraped. (D) The scraped cells are mixed with endocervical drops and smeared by one lateral motion. (E) The slide is immediately placed in 95% ethanol.

When the Pap test is used for the diagnosis of vulvar lesions, the majority of these lesions should be moistened before scraping. A dripping wet compress of sterile water, left for several minutes over a lesion, not only makes it softer for easier scraping but also cleans the lesion by removing most of the nondiagnostic, crusted, or loose and degenerated cellular debris or serum precipitate. After removal of the crust, or the dome of a vesicle, the margins of the ulceration should be energetically scraped with a sharp curette and smeared immediately on an alcohol-moistened slide.

Basic Science

The continuous renewal of the tissues of the body produces an unceasing desquamation of old, diseased, or unwanted cells. The majority of these cells are either lost from the epithelial surface, phagocytized by histiocytes, or destroyed during their passage through the gastrointestinal tract. A small portion of these cells accumulate in natural body cavities and recesses, where they are suspended and preserved in physiologic body secretions or exudates.

The study of these cells for the recognition of alterations produced by normal aging or by various diseases, including carcinomas, was conceived in the nineteenth century (Pouchet, 1847) but came into its own only after the publication of the work of Georges Nicolas Papanicolaou (1883–1962), from whom the name “Pap” test derives (Papanicolaou, 1942).

Clinical Significance

The success and usefulness of the Pap test in gynecology and its expansion to other disciplines (Naib, 1985) have been amply demonstrated in the last 40 years of its use. It has become an important tool for detection of clinically unsuspected diseases, having a high degree of sensitivity and specificity; confirmation of the nature of clinically suspected diseases with minimal trauma; and monitoring the course of diagnosed diseases and their responses to therapy.

In the better institutions, under the sponsorship of the World Health Organization, the classification from 1 to 5 for cervical lesions, which was originally employed successfully by Papanicolaou, has been replaced by a classification similar to that rendered by pathologists examining the histologic sections of tissue biopsies. The numbering system is often abused and misunderstood, and should never be used without a comment on its meaning (Drake, 1984). Class III interpretation, especially, has often served as a convenient way to hide the ignorance or indecision of the cytopathologist.

The variation of the nomenclature of the cytologic reports becomes less significant if the cytopathologist is a full member of a team made up of the clinician, the histopathologist, the colposcopist, and the oncologist. Provided that all speak frequently to one another and use the same language and terminology, they will be able to determine the best treatment for the patient.

The Pap test complements tissue biopsy; the tests do not compete. Each has some advantages and limitations. A Pap

test is inexpensive, rapid, and very simple to obtain and process. It produces no injury to tissues. This allows frequent repetition of cellular sampling, which is especially important in the evaluation of the progressive or posttreatment regression of a disease. It contains samples of cells originating from a wider surface area than that obtained by a biopsy. Areas inaccessible to an easy biopsy (bottom of endocervical glands, endometrial and tubal mucosa, ovarian surface) may exfoliate diagnostic cells to be found in routine genital smears. Certain hormonal states are better evaluated by cytology than by biopsy. The nature of various genital inflammations or infections is often easier to diagnose in a Pap test than in a biopsy. Fungi and parasites are often more visible in a smear. The changes due to irradiation and other forms of therapy are often better evaluated in exfoliated cells than in a histologic sections.

Certain limitations of the Pap smear make biopsy the preferred technique in some situations. The relation of the abnormal cells to the supporting stroma, blood and lymphatic vessels, important in the diagnosis of an invasive carcinoma, cannot be determined by cytology. Other cellular criteria are used for the determination of possible invasion. The location of a lesion cannot be pinpointed by cytology. For example, the same diagnostic squamous cancer cells may originate from the cervix, vagina, or even from the vulva or urethra. The size of a lesion cannot be approximated by cytology. The number of diagnostic cells in a smear often has little relation to the size of the lesion. The nature of a tumor (e.g., poorly differentiated squamous carcinoma as compared to adenocarcinoma or sarcoma) is more difficult to determine. The sample of the cells studied may originate from an unwanted site; for example, rectal, urethra, or vulvar cells may contaminate vaginal smears. The screening of a smear can be time-consuming, and the nature of the lesion is not as obvious in the few scattered cells as in a histologic section. The exfoliated cells may not represent the true nature of the tumor. For example, a neoplasm with mixed components will often exfoliate one type of diagnostic cells.

The value of the Pap test is naturally judged by the number of times it produces the right diagnosis. Its dependability is based on the experience, knowledge, and honesty of the cytopathologist, on the one hand, and the enthusiasm and active cooperation of the clinician, on the other. The clinician is responsible for the choice of the best, rather than the most convenient or cheapest, laboratory to process his smears. Discrepancies in the cytologic diagnosis may be due to:

- Absence of any recognizable diagnostic cells in the smear because of sampling or processing error (*apparent false negative report*)
- Misinterpretation by the cytotechnologist or cytopathologist of the true nature of diagnostic cells present in the smear (*true false negative report*)
- Misinterpretation of the nature of a benign cell rather than of a sampling failure (*false positive report*)

The incidence of these false negative or positive results varies from one laboratory to another. It should be of concern to all. An accurate follow-up of screened patients is essential and should be shared with the laboratory.

References

- Drake M. Nomenclature of precancerous lesions of the uterine cervix. *Acta Cytol* 1984;28:527.
- Naib ZM. Exfoliative cytopathology. 3d ed. Boston: Little, Brown, 1985.
- Papanicolaou GN, Traut HG. Diagnosis of uterine cancer by the vaginal smear. New York: Commonwealth Fund, 1943.
- Pouchet FA. Théorie positive de l'ovulation spontanée et de la fécondation des mammifères et de l'espèce humaine basée sur l'observation de la série animale. Paris: JB Bailliere et Fils, 1847.